

# Lipid Extraction from Wheat Flour Using Supercritical Fluid Extraction<sup>1</sup>

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## ABSTRACT

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Environmental concerns, the disposal cost of hazardous waste, and the time required for extraction in current methods encouraged us to develop an alternate method for analysis of wheat flour lipids. Supercritical fluid extraction (SFE) with carbon dioxide has provided that medium and the method is fully automatic. Crude fats or nonstarch free lipids (FL) were extracted from 4–5 g of wheat flour by an SFE system. To develop optimum conditions for SFE, various extraction pressures, temperatures, and modifier volumes were tried to provide a method that would produce an amount of lipids comparable to those extracted by the AACC Approved Soxhlet Method and the AOCS Official Butt Method using petroleum ether as solvent. Using several wheat flour samples, the best conditions were 12.0 vol% ethanol (10.8 mol%) at 7,500 psi and 80°C to

extract the amount of FL similar to those by the AACC and AOCS methods. Using solid-phase extraction, lipids were separated into nonpolar lipid (NL), glycolipid (GL), and phospholipid (PL) fractions. The mean value of five flours was 1.15% (flour weight, db) by the SFE method, 1.07% by the Butt method, and 1.01% by the Soxhlet method. The SFE-extracted lipids contained less NL and more GL than either the Butt or Soxhlet methods. All three methods extracted lipids with qualitatively similar components. The overall benefit for SFE over the Soxhlet or Butt methods was to increase the number of samples analyzed in a given time, reduce the cost of analysis, and reduce exposure to toxic chemicals.

The quantity and quality of wheat flour lipids are of great interest to cereal scientists because of their nutritional and functional roles in processing wheat-based food products (Chung et al 2002). The traditional and officially approved method for extracting nonstarch free lipids (FL) has been by exhaustive extraction of the lipids using a Soxhlet apparatus with a nonpolar solvent such as hexane, diethyl ether, or petroleum ether (Approved Method 30-25, AACC 2000). Although the Soxhlet method is the AACC Approved Method for extracting lipids, it has a number of disadvantages. Setting up exhaustive extractions can be too laborious and takes considerable time; if not set up properly, there is a potential for fire (or even an explosion), let alone a failed extraction; and if there is a slight water leak in the condensers or in the water circulating tubing, this could lead to flooding in the laboratory. The American Oil Chemists Society Official Method Aa 4-38 (AOCS 2000) discouraged using syphoning methods like Soxhlet because of problems like variable temperature of extraction. Environmental concerns, exposure to toxic vapors, solvent disposal cost of hazardous solvent waste, and time required for extraction have made it imperative to search for a method that would alleviate these concerns.

Supercritical fluid carbon dioxide (SF-CO<sub>2</sub>) (Friedrich et al 1982; King 1993) was used to extract oil from soybeans (Friedrich and List 1982; Friedrich et al 1982; Friedrich 1983); fat from meat products (King et al 1989); and phospholipids (PL) from canola (Temelli 1992; Dunford and Temelli 1995). Wu et al (1990) have defatted corn distillers' dried spent grains with SF-CO<sub>2</sub> and Hopper et al (1995) employed a multiple supercritical fluid extraction

(SFE) approach for food items in their total diet study. SFE technology has been well documented (King 1989, 1993; King and France 1992; King et al 1993; Taylor 1996) and has attracted considerable interest (Latta 1990). This is due to the solvent power of SF-CO<sub>2</sub>, which increases with density at a given temperature (King 1993; Taylor 1996). In addition, diffusivity, the tendency of a dense gas to penetrate the sample matrix, easily increases with temperature (Taylor 1996).

There are no published methods of wheat flour FL extraction using SF-CO<sub>2</sub> as far as we know. Therefore, the objective of this study was to develop a method for extracting FL from wheat flour using an SFE system that would give reproducible results that compare reasonably with that of the Soxhlet or Butt extraction methods, while addressing the above concerns. In this study, we investigated different conditions for extraction pressure (2,500–7,500 lb/sq in. [psi] = 17.2–51.7 MPa), extraction temperature (60–100°C), and modifier concentration (0–19% by volume) to determine the combination of those three variables that would best produce optimum results using one hard red winter (HRW) wheat composite flour. Furthermore, we verified the SFE method using flours from various other wheat classes.

## MATERIALS AND METHODS

### Wheat Flours

Flour K, a composite of HRW wheat cv. Karl grown at six locations in Kansas, was used for development of the SFE method. An additional eight flours including four HRW wheat cultivars (1039, Karl, Larned, and a flour composite designated as Cargill, obtained from Cargill company); two soft red winter (SRW) wheat cultivars (Excel and 2500); and two hard red spring (HRS) wheat cultivars (AcCora and Grandin) were used to verify the SFE method developed and were compared with the Soxhlet method. In addition, three HRW wheat flours (Karl92, Jagger, and Horizon composite), one HRS wheat flour (Kaskaskia), and one SRW wheat flour (Caldwell) were used for FL extraction by three extraction methods including the SFE, Soxhlet, and Butt, and fractionation to nonpolar lipids (NL), glycolipids (GL), and PL (Tweeten et al 1981; Ohm and Chung 1999).

All flour samples were provided by the USDA-ARS Wheat Quality Laboratories; the three HRS wheat flours were provided by the Hard Red Spring and Durum Wheat Quality Lab, Fargo, ND; Caldwell flour was provided by the Soft Wheat Quality Lab, Wooster, OH; and the remaining were provided by the Hard Winter Wheat Quality Lab, Manhattan, KS.

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## Materials

Thin-layer chromatography (TLC) plates and Silica gel G60-coated (250-mm thickness) glass plates were obtained from Aldrich (Milwaukee, WI). Solid-phase extraction (SPE) cartridges were prepacked 1-g Megabond Elut bonded phase silica obtained from Varian (Harbor City, CA) and mounted on a Visiprep SPE vacuum manifold obtained from Supelco Inc. (Belafonte, PA).

## Proximate Analyses

Moisture and protein ( $N \times 5.7$ ) contents of flours were determined according to Approved Methods 44-15A and 46-30, respectively (AACC 2000).

## Soxhlet Extraction

A 10-g (db) sample was used to extract lipids with 150 mL of petroleum ether (Burdick & Jackson Purified Plus Certified Solvent, boiling in the range of 35–60°C) for 16 hr, at a solvent con-

densation rate of 2–3 drops/sec according to AACC Approved Method 30-25 with minor modifications of sample size and extraction time (Chung et al 1980, 1982; Cheung et al 1998).

## Butt Extraction

A sample ( $\approx 4$  g, db) was used to extract lipids with 150 mL of petroleum ether (Burdick & Jackson Purified Plus Certified Solvent, boiling in the range of 35–60°C) for 16 hr at a solvent condensation rate of 2–3 drops/sec (150 mL/min) according to AOCS Official Method Aa 4-38, with minor modifications of sample size and extraction time (AOCS 2000).

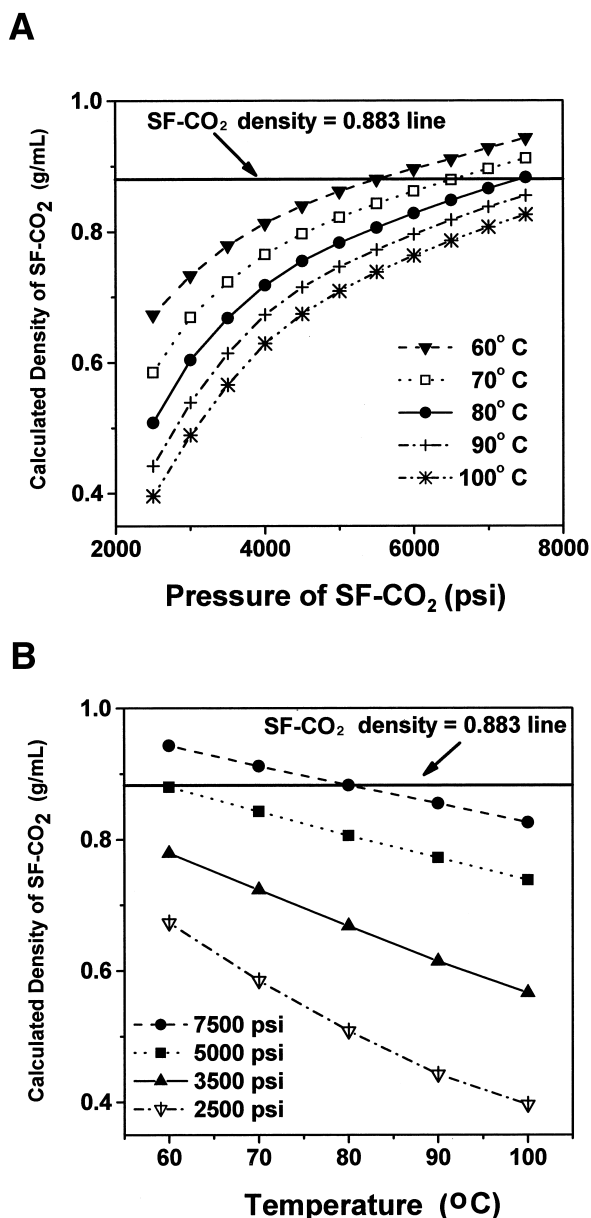
## Supercritical Fluid Extraction (SFE)

SFE was performed with an SFX 3560 automatic extraction system consisting of a 260D (260 mL capacity) pump plus a modifier pump (ISCO, Lincoln, NE). The maximum pressure of the pumps was 7,500 psi (51.7 MPa). To determine the optimum conditions, extractions were performed at various pressures, temperatures, and concentrations of a modifier. First, lipids were extracted from the K flour with only CO<sub>2</sub> beginning at an extraction pressure of 2,500 psi (17.2 MPa), and an extraction temperature of 60°C. Flour ( $\approx 5$  g) was added to a 10-mL cartridge. Pressure was increased systematically by 500-psi increments to 7,500 psi (51.7 MPa), while the temperature was increased systematically from 60 to 100°C at intervals of 10°C.

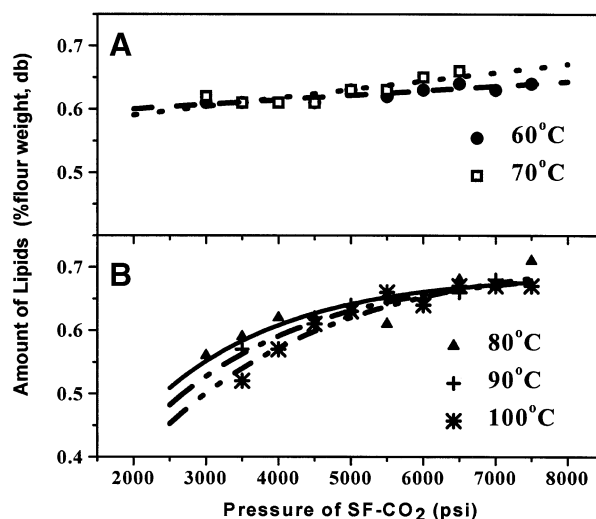
Extracts were concentrated on a rotary evaporator and dried under a stream of nitrogen. The dried lipid was weighed and stored for future analysis in petroleum ether to prevent oxidation. Welding-grade CO<sub>2</sub> (Linweld, Lincoln, NE) was passed through a clean-up column packed with activated charcoal and alumina before entering the pump, according to the purification procedure described by Hopper et al (1995). Absolute ethanol (USP-AAPER Alcohol & Chemical Co., Shelbyville, NE) was used as a modifier and the ethanol reservoir was protected from ambient moisture with a drying tube containing anhydrous calcium sulfate (Drierite, W.A. Hammond Drierite Co., Xenia, OH).

## Thin-Layer Chromatography (TLC)

TLC was used for the separation and identification of lipid components based on the R<sub>f</sub> values of standard lipids including steryl ester (cholesteryl oleate), triglycerides (tristearin, tripalmitin), mono- and diglycerides, free fatty acids (a mixture of stearic, oleic, and linoleic acids), plant sterol mixture, monogalactosyldigly-



**Fig. 1.** A, Variation of density of supercritical fluid SF-CO<sub>2</sub> with pressure in the temperature range of 60–100°C, calculated data based on the report of Pitzer et al (1955). B, Variation of SF-CO<sub>2</sub> density with temperature in the pressure range of 2,500–7,500 psi.



**Fig. 2.** Amount of lipids extracted from K flour with 100% SF-CO<sub>2</sub> in the pressure range of 2,000–8,000 psi at (A) 60 and 70°C and (B) 80–100°C.

cerides, digalactosyldiglycerides, phosphatidyl-ethanolamines, and phostatidylcholines. The TLC was intended mainly for qualitative purposes, and perhaps semiquantitative comparison purposes, as each spot on the plate was at the same concentration (4  $\mu$ L of 20 mg/mL of each sample), so that a reasonable comparison could be made between lanes. Silica gel G60-coated (250-mm thickness) glass plates were freshly activated at 120°C for 30 min each time before use and stored in cabinet containing anhydrous calcium sulfate. Samples were applied to four plates; two were developed in the nonpolar solvent system (I) containing a mixture of hexane, diethyl ether, and methanol (80:20:1, v/v), and the others in the polar solvent system (II) containing a mixture of chloroform, methanol, and water (65:25:4, v/v). After the solvent front had migrated 15 cm, the plates were taken out, dried, and then sprayed with a 0.1M solution of K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> in 50% aqueous sulfuric acid. The plate was heated in an oven at 120°C for 4–8 min. The lipid spots initially turned yellow and then brown and finally black, except for GL that turned a distinct purple before ultimately charring.

### Fractionation of Lipids on Solid-Phase Extraction (SPE)

The lipids extracted by SFE, Soxhlet, or Butt were fractionated on a SPE system using a prepacked 1-g silica gel cartridge column (Rozov and Doulis 2000). Although vacuum could be applied to increase the flow rate of the eluent, generally gravity was sufficient to provide adequate flow. Initially, the cartridges were conditioned with 5 mL of hexane, followed by 5 mL of CHCl<sub>3</sub>. The NL, GL, and PL fractions were successively eluted with 10 mL of CHCl<sub>3</sub> and acetone (4:1, v/v), 15 mL of acetone and methanol (9:1, v/v), and 10 mL of methanol (Ohm and Chung 1999). The purity of the fractions was checked by TLC. Solvents from the fractions were rotary evaporated, transferred to recovery vials using the eluting solvents, and evaporated to dryness at 37°C. The recovery of the lipids was  $\approx$ 100%. Amount of polar lipids was the sum of GL and PL.

### Statistical Analysis

All experiments at given parameters were conducted in duplicate. Statistical analysis was performed using SAS v. 8.0 (SAS Institute, Cary, NC) for analysis of variance (ANOVA) and least significance difference (LSD) test.

## RESULTS AND DISCUSSION

### Effects of Pressure and Temperature on SFE System

Generally, the lipid solubility increased with increases in pressure and temperature (Fig. 1). There was only a slight increase in the solubility of lipid at lower temperatures but there was a greater increase with increasing pressure at higher temperatures. Pressure and temperature determine the density of SF-CO<sub>2</sub>. The data of the density of CO<sub>2</sub> in Fig. 1A and B were computed based on calculations and tables extrapolated from Pitzer et al (1955). The density of SF-CO<sub>2</sub> increased in a nonlinear manner with increasing extraction pressure, and the increase was larger at lower temperatures because, at low pressures, the densities are gas-like but the densities approach that of liquids at high pressure. Liquids are not as compressible as gases, and their densities do not change much with an increase in pressure but the densities approach that of liquids at high pressure. The density of SF-CO<sub>2</sub> decreased with increases in temperature in a linear manner.

The amount of lipids extracted from the K flour with varying pressures are shown in Fig. 2A at 60 and 70°C and in Fig. 2B at 80, 90, and 100°C. Lipid extraction decreased with increasing extraction temperature over the lower extraction pressure range up to 4,000 psi (27.6 MPa) as shown in Fig. 2B. Thus, lipid extraction increased with increasing density of CO<sub>2</sub> gas. Taylor (1996) reported that the solvent power of SF-CO<sub>2</sub> increases with increasing density at a given temperature, and also with an increasing temperature at a given density. In general, extraction increases with gas density to a point, whereas an increase in tem-

TABLE I  
Amount (% flour wt, db) of Lipids Extracted from K Flour at Varying Pressure and Temperature with 100% Supercritical Fluid-CO<sub>2</sub><sup>a</sup>

| Extraction Pressure (psi) <sup>b</sup> | Extraction Temperature (°C) |       |       |       |       |
|--|-----------------------------|-------|-------|-------|-------|
|  | 60                          | 70    | 80    | 90    | 100   |
| 4,500                                  | 0.61                        | 0.61  | 0.62  | 0.62  | 0.61  |
| 5,000                                  | 0.63                        | 0.63  | 0.63  | 0.64  | 0.63  |
| 5,500                                  | 0.62                        | 0.63  | 0.61  | 0.65  | 0.66  |
| 6,000                                  | 0.63                        | 0.65  | 0.65  | 0.65  | 0.64  |
| 6,500                                  | 0.64                        | 0.66  | 0.68  | 0.66  | 0.67  |
| 7,000                                  | 0.63                        | 0.69  | 0.67  | 0.68  | 0.67  |
| 7,500                                  | 0.64                        | 0.62  | 0.71  | 0.67  | 0.67  |
| Mean                                   | 0.63                        | 0.64  | 0.65  | 0.65  | 0.65  |
| SD                                     | 0.010                       | 0.025 | 0.033 | 0.020 | 0.022 |

<sup>a</sup> Average of two replicates.

<sup>b</sup> Pounds/square inch (145 psi = 1 MPa).

TABLE II  
Amount (% flour wt, db) of Lipids Extracted from K Flour with SF-CO<sub>2</sub> and Ethanol Modifier<sup>a</sup>

| Modifier% (v/v) | Extraction Temperature (°C) |      |      |      | At a Given Modifier Level |                 |
|-----------------|-----------------------------|------|------|------|---------------------------|-----------------|
|                 | 70                          | 80   | 90   | 100  | Mean <sup>b</sup>         | SD <sup>c</sup> |
| 11.0            | 0.99                        | 1.00 | 0.95 | 0.95 | 0.97D                     | 0.03            |
| 12.0            | 1.04                        | 1.04 | 1.02 | 1.02 | 1.03C                     | 0.01            |
| 12.5            | 1.06                        | 1.05 | 1.04 | 1.03 | 1.05C                     | 0.01            |
| 12.8            | 1.07                        | 1.06 | 1.05 | 1.04 | 1.06C                     | 0.01            |
| 13.0            | 1.10                        | 1.08 | 1.08 | 1.05 | 1.08C                     | 0.03            |
| 15.0            | 1.15                        | 1.15 | 1.17 | 1.15 | 1.16B                     | 0.01            |
| 17.0            | 1.18                        | 1.18 | 1.27 | 1.24 | 1.22A                     | 0.05            |
| 19.0            | 1.21                        | 1.21 | 1.30 | 1.34 | 1.27A                     | 0.07            |

<sup>a</sup> Average of two replicated extracts at a given pressure of 7,500 psi (51.7 Mpa).

<sup>b</sup> Mean values of four temperatures at a given modifier concentration with different letters were significantly different ( $P < 0.001$ ).

<sup>c</sup> Standard deviation ( $n = 8$ ).

perature at a given pressure reduces density and thus the polarity and extractability. At higher pressures, an increase in temperature had little effect on the amount of lipids extracted (Fig. 2B). The analysis of variance showed a highly significant pressure effect but an insignificant temperature effect on the amount of lipids extracted. The LSD test showed that the mean quantity values of lipids extracted at pressures between 4,500 and 7,500 psi were significantly different only at higher temperatures (Table I). The amount of lipids extracted with CO<sub>2</sub> alone was greatest at 7,500 psi/80°C (0.71% db, Table I) at the CO<sub>2</sub> density of 0.883 g/mL (Fig. 1A), whereas the density was highest at 7,500 psi/60°C (0.943 g/mL). At the highest density, the amount of lipids extracted was only 0.64% (db). Even though the CO<sub>2</sub> density at 7,500 psi/80°C is similar to that at 5,500 psi/60°C (Fig. 1A), the amount of lipids

extracted was higher at 7,500 psi/80°C than at 5,500 psi/60°C (0.71 vs. 0.62%, respectively). Therefore, gas density is not the only factor in controlling extraction of lipids from the flour matrix; the enhancement in the vapor pressure of the lipid volutes must also be considered.

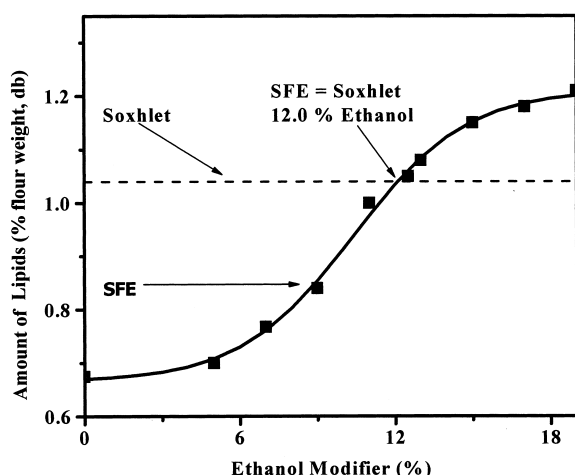
### Effect of Modifier Concentration Variation

The average amount of FL extracted from 10 g (db) of the K flour averaged 1.04% (db) using AACC Approved Method 30-25 of the Soxhlet extraction method using petroleum ether. Another sample of Karl wheat flour averaged 0.98% (db) with a standard deviation of 0.011 ( $n = 7$ ). These values are typical of Soxhlet extractions of a large number of wheat flour samples we have analyzed in this laboratory but the data are not included here.

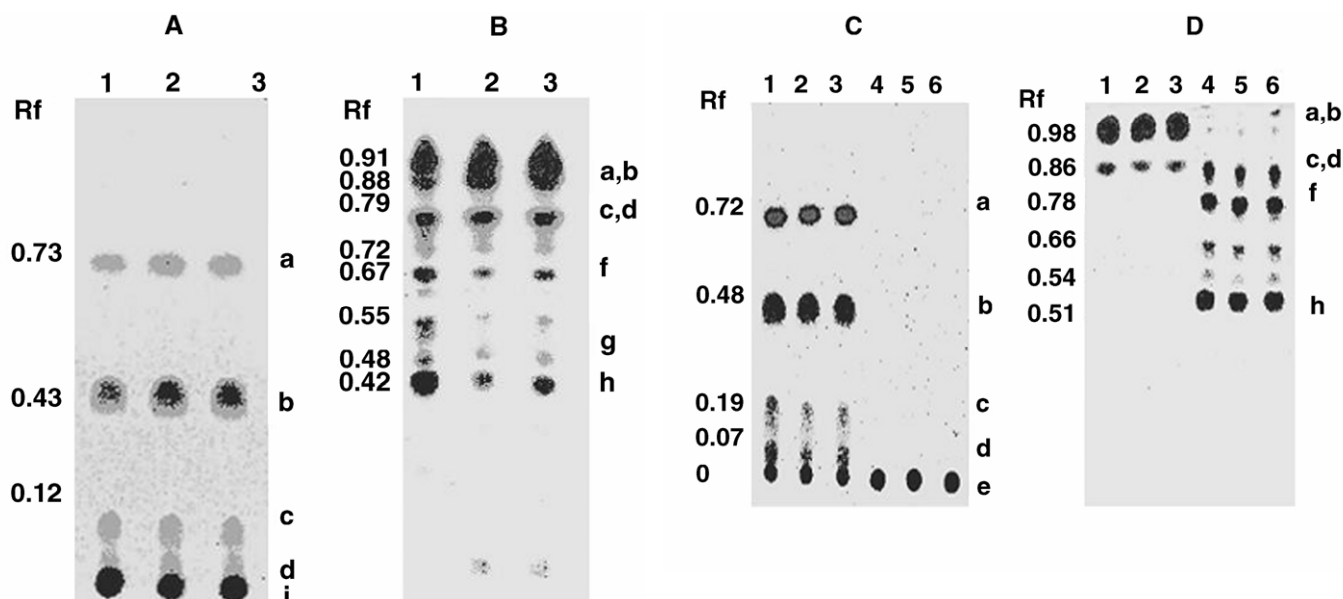
It was obvious that the amount of lipids (0.61–0.71%) (Table I) extracted by SFE with CO<sub>2</sub> only could not match the amount of lipids extracted by Soxhlet. Because SF-CO<sub>2</sub> is not sufficiently polar enough to extract the polar lipids, methanol or ethanol was added in a precise amount to SF-CO<sub>2</sub> using a second syringe pump as a modifier or co-solvent to increase the polarity of SF-CO<sub>2</sub>.

Methanol was the initial modifier tried to increase the extraction of lipids. However, it proved difficult and much too time-consuming to remove the methanol-water mixture formed during lipid extraction using the rotary evaporator. Because Dunford and Temelli (1995) used ethanol for extracting PL from canola, we tried a binary mixture of CO<sub>2</sub> plus absolute ethanol as a modifier. Absolute ethanol was used to control the amount of water added to the extraction mixture. Water changes the polarity and also makes concentrating the lipid extracts to near dryness more difficult. At a given pressure of 7,500 psi, increasing modifier concentration increased lipid extractability at all four temperatures (Table II). In Fig. 3, the intersection of the line representing the amount of lipids obtained in the Soxhlet extraction would correspond to the percentage of modifier necessary in the SFE to resemble the amount of lipids by Soxhlet extraction. The amount of lipid extracted by SF-CO<sub>2</sub> containing 12.0% ethanol modifier closely resembled that by the AACC Approved Soxhlet method.

ANOVA indicated that modifier and modifier-by-temperature interaction effects were significant ( $P < 0.001$ ) at the extraction



**Fig. 3.** Amount of lipids extracted from K flour by SFE as a function of modifier concentration. The point of intersection in the SFE curve at the Soxhlet value represents the corresponding modifier concentration necessary to match the two methods.



**Fig. 4.** Qualitative TLC of Karl92 flour lipids. **A**, Unfractionated lipids developed in a nonpolar solvent system (I) (hexane:diethyl ether:methanol, 80:20:1, v/v). **B**, Unfractionated lipids developed in a polar solvent system (II) of (chloroform:methanol:water, 65:25:4, v/v). **C**, Developed in solvent system (I) with lanes (1–3) of NL and (4–6) of GL. **D**, Developed in solvent system (II) with lanes (1–3) of NL and (4–6) of GL. Lanes (1, 4) lipids extracted by SFE; lanes (2, 5) lipids extracted by Butt extraction; lanes (3, 6) lipids extracted by Soxhlet method. TLC bands identified by standard lipids: a, steryl esters; b, triglycerides; c, free fatty acids; d, di- and monoglycerides; f, monogalactosyldiglycerides; g, phostatidylethanolamines; h, digalactosyldiglycerides; i, all polar lipid fractions, containing GL and PL.

pressure of 7,500 psi. The LSD test showed that mean amounts of lipids extracted using a modifier at concentrations of 12.0–13% (v/v) were not statistically different. And furthermore, there were no significant temperature-by-modifier interaction effects in the modifier concentration range of 12.0–13% (v/v) and extraction temperatures of 70–100°C.

The FL extracted from 10 g (db) of the K flour averaged 1.04% (db) by Soxhlet extraction and also by SFE at 7,500 psi/80°C or 70°C with 12.0% (v/v) absolute ethanol. Thus, the extraction conditions of 7,500 psi/80 and 12.0% by volume (10.8 mol%) ethanol modifier were optimal for the SFE method of FL extraction from the K flour (Table II, Fig. 3).

#### SFE Method Validation

*Comparison of Soxhlet and SFE.* Karl flour (not the K flour) gave an average lipid amount of 0.98% (SD 0.011,  $n = 7$ ) by Soxhlet and 1.03% (SD 0.045,  $n = 8$ ) by the optimized SFE of 7,500 psi, 80°C, and 12.0% by volume (10.8 mol%) ethanol modifier.

*Tests on other samples.* Eight flours from three wheat classes were selected for testing the SFE method, and lipids were extracted

by three methods (Table III). The optimum parameters, obtained from the K flour studies, used to perform the extractions were an extraction chamber pressure of 7,500 psi; extraction chamber temperature of 80°C; the restrictor temperature of 90°C; a modifier 12.0% by volume (10.8 mol%); a flow rate of 3.0 mL/min (2.685 g/min); a maximum extraction fluid volume of 60.0 mL; and a dynamic extraction time of 20 min. There were noticeable differences in lipid extraction by the SFE (CO<sub>2</sub> alone) method between flour samples. The SFE method (SF-CO<sub>2</sub> plus 12.0%, v/v, ethanol) data were similar to those for the Soxhlet extraction. In all cases, extractions with neat CO<sub>2</sub> were significantly lower than with a modifier, which indicates that a polar solvent was required to solubilize the polar lipids.

#### Comparison of SFE, Soxhlet, and Butt Extraction

For a comparison, we extracted five additional flours by SFE (with 12% ethanol), Soxhlet, and Butt extraction methods (Table IV). Then the extracted lipids were fractionated to NL, GL, and PL, expressed as % of lipids. The analysis of variance showed the significant effects of extraction methods, flours, and their interac-

TABLE III  
Amount (% flour wt, db) of Lipids Extracted from Wheat Flour Samples by Three Methods<sup>a</sup>

| Wheat Class                    | Flour Samples       | Soxhlet | SF-CO <sub>2</sub> Only | SFE                                      |
|--------------------------------|---------------------|---------|-------------------------|--|
|                                |                     |         |                         | SF-CO <sub>2</sub> + 12.0% (v/v) Ethanol |
| HRW                            | 1039                | 0.93    | 0.62                    | 0.94                                     |
|                                | K                   | 1.04    | 0.70                    | 1.04                                     |
|                                | Karl                | 0.98    | 0.67                    | 0.94                                     |
|                                | Larned              | 0.97    | 0.83                    | 0.99                                     |
|                                | Cargill (Composite) | 1.00    | 0.88                    | 1.20                                     |
| HRS                            | AcCora              | 0.94    | 0.76                    | 0.94                                     |
|                                | Grandin             | 0.89    | 0.69                    | 0.97                                     |
| SRW                            | Excel               | 1.10    | 0.83                    | 1.03                                     |
|                                | 2500                | 0.99    | 0.82                    | 1.06                                     |
| Mean ( $n = 18$ ) <sup>b</sup> |                     | 0.98A   | 0.76B                   | 1.01A                                    |

<sup>a</sup> Average of two replicates. Supercritical fluid extraction (SFE) conditions included 7,500 psi of extraction pressure and 80°C of extraction temperature and 60 mL of extraction volume.

<sup>b</sup> Mean values of nine wheat flour samples with different letters were significantly different ( $P < 0.001$ ).

TABLE IV  
Amount (% flour wt, db) of Lipids Extracted by Three Methods and Their Fractions (% Lipids) by SPE Method

| Wheat Samples           | Extraction Method | Amount of Lipids (% flour wt, db) | % Lipids <sup>a</sup> |       |      |
|-------------------------|-------------------|-----------------------------------|-----------------------|-------|------|
|                         |                   |                                   | NL                    | GL    | PL   |
| Karl92 (HRW)            | SFE               | 1.08                              | 64.5                  | 30.2  | 5.2  |
|                         | Soxhlet           | 0.91                              | 83.8                  | 12.4  | 3.9  |
|                         | Butt              | 0.88                              | 80.1                  | 15.3  | 4.6  |
| Jagger (HRW)            | SFE               | 0.90                              | 61.7                  | 32.3  | 6.2  |
|                         | Soxhlet           | 0.88                              | 76.9                  | 21.2  | 1.9  |
|                         | Butt              | 0.89                              | 78.3                  | 15.5  | 6.2  |
| Kaskaskia (HRS)         | SFE               | 1.43                              | 64.6                  | 30.6  | 4.8  |
|                         | Soxhlet           | 1.24                              | 85.0                  | 12.1  | 2.8  |
|                         | Butt              | 1.44                              | 80.9                  | 14.1  | 4.9  |
| Caldwell (SRW)          | SFE               | 1.19                              | 63.3                  | 31.8  | 4.9  |
|                         | Soxhlet           | 1.05                              | 89.8                  | 7.9   | 2.3  |
|                         | Butt              | 1.04                              | 81.7                  | 12.3  | 5.9  |
| Horizon (HRW composite) | SFE               | 1.16                              | 73.6                  | 21.6  | 4.7  |
|                         | Soxhlet           | 0.97                              | 92.5                  | 6.5   | 1.0  |
|                         | Butt              | 0.99                              | 81.3                  | 13.7  | 4.8  |
| Mean <sup>b</sup>       | SFE               | 1.15A                             | 65.5C                 | 29.3A | 5.2A |
|                         | Soxhlet           | 1.01B                             | 85.6A                 | 12.0B | 2.4B |
|                         | Butt              | 1.07B                             | 80.3B                 | 14.2B | 5.4A |

<sup>a</sup> Overall mean recovery of lipid fractionation was 95% (SD 7%). NL, nonpolar lipids; GL, glycolipids; PL, phospholipids.

<sup>b</sup> Mean values of five flours with different letters were significantly different ( $P < 0.05$ ).

tions on the amount of lipids extracted and also lipid composition, including NL and GL but not PL. The extraction method effects were much more significant than the flour effects. Mean values of five flours showed that the Soxhlet and Butt methods could extract similar amounts of lipids and their fractionation showed similar GL contents (% lipids). However, the NL contents (% lipids) differed depending on the lipid extraction method (Table IV). Between the three methods, the SFE (with 12% ethanol) was statistically different from the Soxhlet and Butt extraction methods. Significantly higher lipid amounts extracted by the SFE method were due to substantially more GL from the matrix than the two exhaustive Soxhlet and Butt extraction methods.

### Thin-Layer Chromatography (TLC)

Lipids extracted by SFE, Butt, and Soxhlet methods (Lanes 1, 2, and 3 in Fig. 4) showed qualitatively similar components for NL and polar lipids. However, the lipid composition differed for the SFE-extracted lipids from lipids extracted by the Butt and Soxhlet methods due to mainly quantitative differences in polar fraction, specifically GL including monogalactosyldiglycerides (f) and digalactosyldiglycerides (h) (Fig. 4B). The NL and GL fractions obtained by the SPE system showed similar NL composition (lanes 1–3, Fig. 4C and D) as well as GL composition (lanes 4–6, Fig. 4C and D). Therefore, the composition of NL or GL fractions was not affected by the extraction methods. However, the SFE (with 12% ethanol) extracted lipids with more GL and less NL than the Butt and Soxhlet methods.

### Savings by SFE Method

The SFE method cost per sample proved to be less than that using a Soxhlet or Butt method. SFE with a modifier required only 7.4 mL of ethanol per extraction, compared with 150 mL of petroleum ether for a Soxhlet or Butt extraction. Hence, a 20:1 reduction in the use of organic solvent was achieved by using SFE. In addition to reducing costs, ethanol is much less of a fire hazard and is less toxic. Thus, there was a reduction in the cost of solvent and in the disposal costs using SFE with a modifier. Several hundred SF analyses may be performed with a 70-lb tank of industrial grade CO<sub>2</sub> at a cost of about \$20/tank. The SFE method calls for a 20-min extraction using a total solvent (fluid) volume of 60 mL plus  $\approx$ 10 min for flushing the system and refilling the pumps. The method is fully automatic and will perform 24 SFE extractions in 12 hr compared with 8 extractions for Soxhlet (in our laboratory setup) per day. It was possible to perform 48 SFE extractions in a 24-hr period. Therefore, SFE method would reduce extraction time almost sixfold.

This study showed that the SFE method could save time, cost, and solvent usage, and is more environmentally friendly. The study also showed that a polar modifier was required to successfully extract the FL (NL and polar lipids) from wheat flour by SFE when compared with the lipid extracted by the Soxhlet and Butt extraction methods.

## CONCLUSIONS

The data showed that SFE provides a method for extracting the nonbound or FL from wheat flour. The fractionation and TLC show that the individual lipid extracted was qualitatively similar, but not quite the same quantitatively. However, the other reasons (cost, time, and environmental concerns) for development of the method have been met.

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